# Long-term Treatment of Malignant Gliomas with Intramuscularly Administered Polyinosinic-Polycytidylic Acid Stabilized with Polylysine and Carboxymethylcellulose: An Open Pilot Study

Andres M. Salazar, M.D., Hilton B. Levy, Ph.D., Steven Ondra, M.D., Meir Kende, Ph.D., Barbara Scherokman, M.D., Douglas Brown, M.D., Hernando Mena, M.D., Norman Martin, M.D., Karen Schwab, Ph.D., Daniel Donovan, M.D., David Dougherty, M.D., Morris Pulliam, M.D., Mark Ippolito, M.D., Maria Graves, R.N., Herbert Brown, Alex Ommaya, M.A.

Uniformed Services University of the Health Sciences (AMS, BS, KS, DDou, MG, HB, AO), National Institute of Allergy and Infectious Diseases (HBL), Walter Reed Army Medical Center (SO, DB, NM, DDon), United States Army Medical Research Institute for Infectious Diseases (MK), Armed Forces Institute of Pathology (HM), and National Naval Medical Center (MP, MI), Washington, District of Columbia

**POLYINOSINIC-POLYCYTIDYLIC ACID STABILIZED with polylysine and carboxymethylcellulose (poly-ICLC) (10–50 mcg/kg, administered intramuscularly one to three times weekly) was given for ≤56 months to 38 patients with malignant gliomas. There was minimal or no toxicity. Twenty of 30 patients (66%) receiving at least twice weekly poly-ICLC showed regression or stabilization of gadolinium-enhancing tumor, as revealed by magnetic resonance imaging (median = 65% volume decrease). All but one patient with anaplastic astrocytomas who received continuous poly-ICLC remain alive, with a median progression-free survival of 54 months from diagnosis. Median Kaplan-Meier survival is 19 months for patients with glioblastomas who receive at least twice weekly poly-ICLC treatments. Tumor response was associated with 2′,5′-oligoadenylate synthetase activation ($P = 0.03$) but not with serum interferon. We hypothesize clinical activation by poly-ICLC of a basic host tumor suppressor system. Prolonged, quality survival with tumor stabilization or regression confirmed by magnetic resonance imaging for most patients with anaplastic astrocytomas and glioblastomas suggests that more extensive laboratory and controlled clinical studies are warranted. The concept of long-term, broad spectrum stimulation of host defenses with nontoxic, inexpensive double-stranded ribonucleic acids, such as low-dose poly-ICLC, may be applicable to the treatment of other malignancies.** (Neurosurgery 38:1096–1104, 1996)

Key words: dsRNA, Interferon, Malignant glioma, Oligoadenylate, Poly-ICLC, Tumor suppressors

Postsurgical treatment of malignant gliomas has been disappointing. Radiation therapy increases median survival from ~6 to 9 months for glioblastomas (GBMs) and 24 months for Grade III anaplastic astrocytomas (AAs) (17, 41); traditional chemotherapy adds only modestly to that (13, 35, 36). More aggressive combined chemotherapy has recently been shown to increase the 4-year progression-free survival of AAs to ~42% (18). Biological agents, such as interferon (IFN), have shown some promise but have not lived up to original expectations (24).

Polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) (23) is a double-stranded ribonucleic acid (dsRNA) that was used as an IFN inducer at high doses (≤300 mcg/kg, administered intravenously) in short-term cancer trials years ago. This gave mixed results and moderate toxicity (7, 11, 15, 20, 29, 30, 39). However, lower-dose (10–50 mcg/kg) poly-ICLC results in a broader host-defense stimulation, including T-cell and NK-cell activation, cytokine release (IFN-$\alpha$, -$\beta$, and -$\tau$, interleukins [ILs], corticosteroids, and tumor necrosis factor [TNF]), a potent adjuvant effect, and a specific antiretroviral effect mediated by the 2′,5′-oligoadenylate synthetase (OAS) and $P_{68}$ kinase nuclear enzyme systems (2, 8, 21, 22, 37, 38). Poly-IC also preferentially decreases tumor protein synthesis in vivo (19).

This background, in addition to our experience with poly-ICLC in multiple sclerosis and human immunodeficiency virus (HIV) infection, led us to a pilot trial in malignant gliomas (1, 32). The tolerance and toxicity of low-dose poly-ICLC has been described in previous studies. In preparation for a possible controlled efficacy study, the intent of the present pilot study was to further evaluate toxicity and explore the possible usefulness of several low-dosage regimes of poly-ICLC in patients with malignant gliomas who were otherwise receiving standard radiation with or without chemotherapy.

## PATIENTS AND METHODS

We report on the first 38 Walter Reed Army Medical Center patient volunteers referred for study (*Table 1*). Entrance criteria were as follows: 1) biopsy-proven malignant glioma, 2) minimum Karnofsky score of 50, 3) at least 1 month after having completed radiation therapy, and 4) signed volunteer informed consent. There were 18 newly diagnosed Grade IV patients (patients with GBMs) and 11 newly diagnosed Grade III patients (patients with AAs), most of whom had residual tumor at study entry (*Table 1*). In addition, there were nine patients with recurrent GBMs or AAs who had progressive disease as revealed by radiography at study entry (*Table 1*). The median time between diagnosis or end of radiation therapy and entrance into the study was 7 and 3 months for patients with AAs and 5 and 2 months for patients with GBMs, respectively. The median age was 58 years for patients with GBMs and 32 years for patients with AAs; there were 31 men and 7 women. Median gadolinium-enhancing tumor volume, as revealed by magnetic resonance imaging (MRI), was 19 cc (range, 0–88 cc) at study entry (postoperative and ≥1-mo postradiation therapy) (*Table 1*). The median Karnofsky score at entry was 90 (range, 60–100) for patients with AAs, 80 (range, 60–100) for patients with GBMs, and 80 (range, 50–90) for patients with recurrent gliomas. Two patients with GBMs who dropped out of the study early (one because of lomustine [CCNU] side effects at 8 weeks and the other for personal reasons at 1 week) are not included in the report. The study population was drawn from ~60 patients with AAs or GBMs who had been admitted to Walter Reed Army Medical Center during the accession period. Among those, 18 declined to participate or were not referred by their physicians and seven did not meet the Karnofsky score requirement.

Patients were initially entered sequentially into four groups of five patients each, given poly-ICLC doses of 10 and 50 mcg/kg, administered intramuscularly once a week, and 10 and 20 mcg/kg, administered twice a week, respectively (*Table 1*). Additional patients were then entered into a fifth group treated with 20 mcg/kg two or three times per week. Each patient or a family member was taught to give the injections at home and to maintain a regular treatment diary. All but one patient had completed a full course of radiation therapy (median = 6000 rads) a median of 3 months before entry. That one patient (Patient 10), who had an AA, had refused radiation therapy. Twenty patients also concurrently received one to seven doses of CCNU (120 mg/M2, every 6 wk) during the first 12 months. Two patients (Patients 27 and 38) had received carmustine before study entry. Anticonvulsants and dexamethasone were administered as indicated.

Follow-up included standardized neurological examinations, electroencephalography, and MRI performed on a 1.5-tesla General Electric Signa scan at 3- to 6-month intervals. Lesion volume was measured on a computerized light table (SigmaScan; Jandel Scientific) and with an image analysis program (Medvision 1.4). Tumor partial anatomic response (PR) was defined as ≥50% gadolinium-enhancing lesion volume decrease, as revealed by MRI on a stable or decreasing steroid dose; 100% decrease was classified as complete response (CR) (*Table 1*). Subsequent scans generally were not performed to confirm a response or its duration until the next scheduled protocol scan, usually 6 months later (see Results) (*Fig. 1*). Patients were classified as stable (S) if lesion volume decreased by <50% or did not increase by ≥20% in the first 6 months of study. Neuroradiologists reading the scans and the technician measuring the scans were unaware of the clinical statuses of the patients, although they were aware of patient participation in the study. Measurements on a given scan were ascertained without reference to prior scans or data. Special laboratory data included lymphocyte subsets by flow cytometry, serum OAS activity (radioimmunoassay; Eiken Chemical Company, RIA), serum IFN (vesicular stomatitis virus bioassay), TNF, IL-2, IL-6, and neopterin. These were performed at baseline, 1, 2, and 7 days later, at 3 months, and generally every 6 months thereafter. The median survival was estimated using Kaplan-Meier survival analysis.

## RESULTS

Poly-ICLC was exceptionally well tolerated, with little or no toxicity. Quality of life was preserved in most patients. The

**TABLE 1. Polyinosinic-Polycytidylic Acid Stabilized with Polylysine and Carboxymethylcellulose Used in the Treatment of Malignant Gliomas: Patient Evaluation[a]**

| Diagnosis | Patient Number | Age (yr) | Prognostic Class[b] | Dosage[c] (mcg/kg/wk) | Mo on PICLC | CCNU Cycles | Survival (mo)[d] | Mo to Progression[e] | OAS Change (%)[f] | Tumor Size[g] (cc) | Best Tumor Change (%) | Tumor Response |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Anaplastic Astrocytoma* | | | | | | | | | | | | |
| | 1 | 23 | 1 | 10 × 1 | 24 | 7 | 76 | NP | 310 | 5 | −92 | PR |
| | 2 | 31 | 3 | 10 × 2 | 54 | 1 | 61 | 50 | 334 | 74 | −94 | PR |
| | 3 | 34 | 1 | 10 × 2 | 52 | 7 | 61 | NP | 1300 | 0 | 0 | S |
| | 4 | 30 | 1 | 10 × 2 | 52 | 5 | 61 | NP | – | 16 | −100 | CR |
| | 5 | 36 | 1 | 10 × 2 | 43 | 5 | 50 | NP | – | 0 | 0 | S |
| | 6 | 54 | 2 | 10 × 2 | 5[i] | 4 | 46 | NP | – | 8 | −40 | S |
| | 7 | 28 | 1 | 20 × 2 | 48 | 4 | 65 | NP | 900 | 2 | −100 | CR |
| | 8 | 27 | 3 | 20 × 2 | 45 | 4 | 54 | NP | 400 | 28 | −98 | PR |
| | 9 | 46 | 3 | 20 × 2 | 44 | 5 | 55 | NP | 1600 | 22 | −73 | PR |
| | 10 | 32 | 3 | 20 × 2 | 39 | 1 | 44 | NP | 1335 | 14 | −99 | PR |
| | 11[h] | 40 | 1 | 20 × 3 | | 0 | 34 | 30 | 1400 | 3 | −50 | PR |
| | Median | 32 | | | | | 55 | 54 | | | | |
| *Glioblastoma* | | | | | | | | | | | | |
| | 12[h] | 43 | 3 | 10 × 1 | 12[i] | 6 | 17 | 11 | 370 | 23 | 129 | P |
| | 13[h] | 60 | 5 | 10 × 1 | 8[i] | 4 | 15 | 11 | 76 | 25 | 118 | P |
| | 14[h] | 62 | 5 | 50 × 1 | 9 | 3 | 15 | – | 43,000 | 36 | −19 | S |
| | 15[h] | 57 | 5 | 50 × 1 | 4 | 2 | 10 | NP | – | 3 | – | – |
| | 16[h] | 64 | 5 | 50 × 1 | 5 | 3 | 11 | 10 | 161 | 26 | 173 | P |
| | 17[h] | 67 | 5 | 50 × 1 | 4 | 0 | 10 | 10 | 50 | 9 | 223 | P |
| | 18[h] | 36 | 3 | 20 × 2 | 16 | 4 | 26 | 19 | 775 | 38 | −34 | S |
| | 19[h] | 50 | 5 | 20 × 2 | 15 | 0 | 24 | 15 | 11,467 | 28 | 214 | P |
| | 20 | 42 | 4 | 20 × 2 | 35 | 0 | 48 | NP | 1060 | 7 | −100 | CR |
| | 21[h] | 70 | 6 | 20 × 2 | 11 | 0 | 19 | 15 | 2250 | 65 | −14 | S |
| | 22[h] | 36 | 3 | 20 × 2 | 20 | 0 | 26 | 22 | – | 9 | −25 | S |
| | 23[h] | 61 | 5 | 20 × 2 | 8 | 0 | 12 | 8 | 842 | 8 | 195 | P |
| | 24 | 32 | 3 | 20 × 3 | 31 | 0 | 39 | 21 | – | 6 | −65 | PR |
| | 25[h] | 35 | 3 | 20 × 3 | 10 | 0 | 15 | – | – | 15 | −66[j] | PR |
| | 26[h] | 70 | 6 | 20 × 3 | 8 | 0 | 12 | – | 414 | 59 | 17 | S |
| | 27[h] | 58 | 5 | 20 × 3 | 7 | BCNU | 19 | 14 | 567 | 39 | 47 | P |
| | 28[h] | 60 | 6 | 20 × 3 | 5 | 0 | 8 | 6 | 2000 | 19 | 130 | P |
| | 29 | 22 | 3 | 20 × 3 | 34 | 0 | 41 | NP | – | 3 | 0 | S |
| | Median | 58 | | | 10 | | | | | | | |
| Recurrent Glioblastoma or Anaplastic Astrocytoma | | | | | | | | | | | | |
| | 30[h] | 25 | 4 | 10 × 1 | 4 | 2 | 40 (6) | – | 87 | 57 | – | – |
| | 31[h] | 26 | 4 | 10 × 2 | 6[i] | 4 | 37 (18) | – | 1294 | 70 | 475 | P |
| | 32[h] | 35 | 3 | 10 × 2 | 4 | 2 | 68 (7) | – | – | 88 | – | P |
| | 33 | 33 | 3 | 20 × 2 | 35 | 5 | 68 (47) | NP | 4900 | 28 | −100 | CR |
| | 34 | 41 | 3 | 20 × 2 | 35 | 0 | 73 (37) | NP | 3500 | 24 | −50[j] | PR |
| | 35[h] | 62 | 2 | 20 × 2 | 9[i] | 0 | 35 (10) | – | 404 | 1 | 200 | P |
| | 36[h] | 60 | 5 | 20 × 2 | 10[i] | 0 | 32 (17) | – | 193 | 29 | 131 | P |
| | 37[h] | 30 | 4 | 20 × 3 | 11 | 0 | 105 (12) | – | 1520 | 13 | 390 | P |
| | 38[h] | 42 | 4 | 20 × 3 | 9[i] | BCNU | 67 (12) | – | 980 | 18 | – | P |
| | Median | 35 | | | 11 | | 68 (12) | – | | | | |

[a] PICLC, polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose; CCNU, lomustine; OAS, oligoadenylate synthetase; NP, no progression; PR, partial response; S, stable; CR, complete response; P, progression; –, not applicable; BCNU, carmustine.

[b] Prognostic class presented by Curran et al. (6) (see text and Ref. 25).

[c] Poly-ICLC administered intramuscularly.

[d] Survival or follow-up time (mo from diagnosis or from recurrence [in parentheses]).

[e] Months from diagnosis to radiographically determined progression.

[f] Maximum change in serum oligoadenylate synthetase in response to poly-ICLC treatment.

[g] Gadolinium-enhancing lesion volume at study entry (postoperative and after radiation therapy).

[h] Patient died.

[i] Intermittent treatment.

[j] Percent decrease of enhancing lesion after initial increase at 3 to 6 months (see Fig. 1).

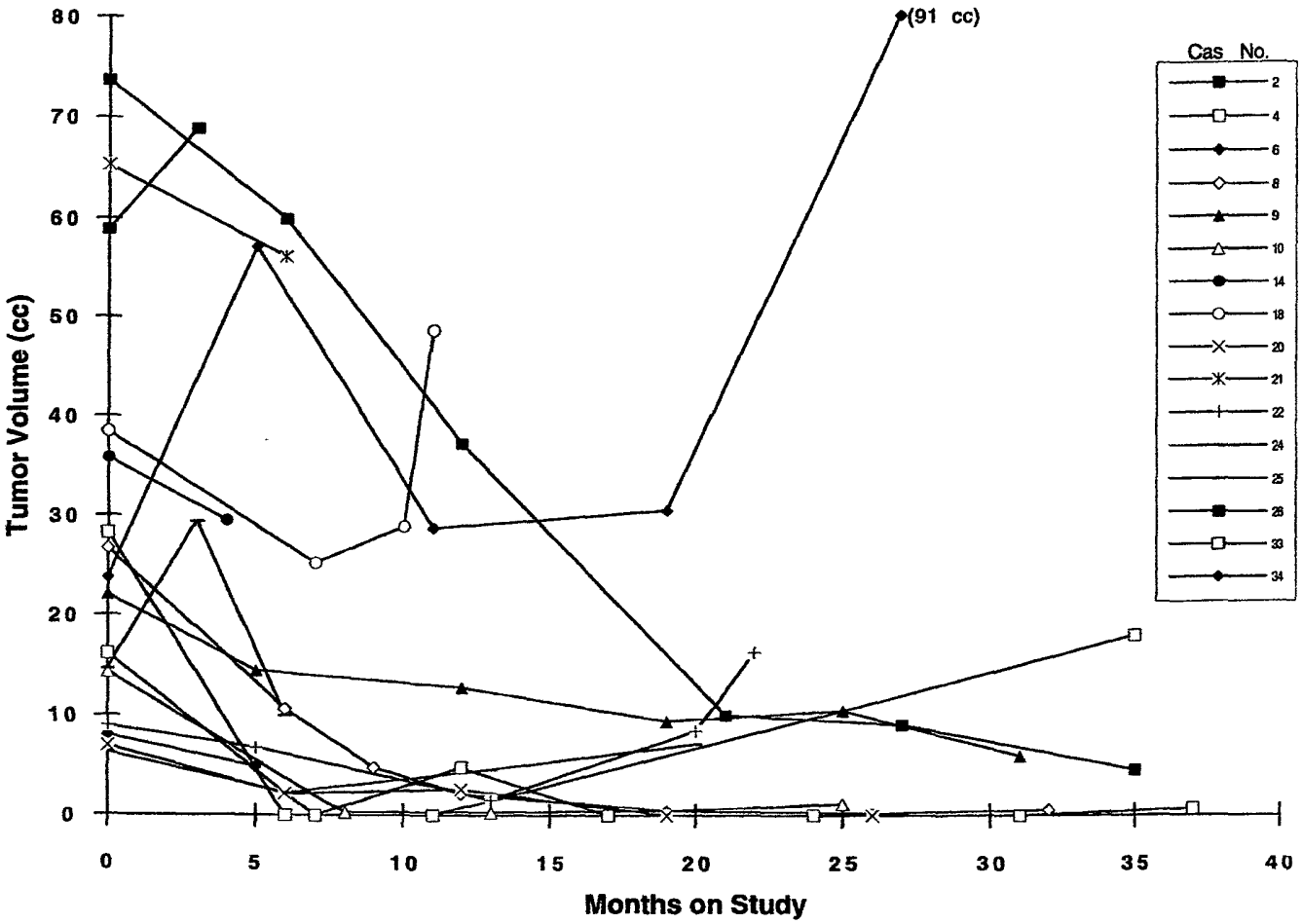


**FIGURE 1. Poly-ICLC in malignant gliomas. Gadolinium-enhancing tumor volume change over time, as revealed by MRI in patients with tumors >5 cc who were responding or stable with the administration of poly-ICLC. Seven additional responders with smaller tumors are not shown.**

most common complaint was of mild, temporary discomfort at the injection site. Several patients complained of an occasional transient fever or malaise after injection (greater in the 50 mcg/kg group), but this usually resolved after the first few treatments.

All but one patient with Grade III AAs who received continuous poly-ICLC remain alive a median of 55 months (range, 42–74 mo) from diagnosis (*Table 1*). Median progression-free survival is now 54 months. One patient who had an AA died at 34 months, after recurrence at 30 months. Living patients with AAs are all well off of corticosteroids, except for two patients, one with biopsy-proven radiation necrosis at 42 months from diagnosis (Patient 9) and one with tumor recurrence at 52 months from diagnosis (Patient 2). Another patient with an AA (Patient 10) who refused radiation therapy is well 42 months from diagnosis. Only 3 of 18 patients with GBMs remain alive. The median Kaplan-Meier survival for patients with GBMs who are receiving at least twice weekly continuous therapy is 19 months (95% confidence interval, 9–29 mo) (*Fig. 2, Table 1*). However, median survival was 11 months for the six patients with GBMs who received only once weekly or intermittent poly-ICLC and 17 months for all patients with GBMs. Most deaths were caused by tumor progression or by recurrence after response; 15 patients with GBMs died, and two of those deaths (Patients 15 and 25) were caused by pulmonary emboli without evidence of tumor progression. Patients with recurrent gliomas now have median survival or follow-up of 12 months from time of recurrence or 68 months from diagnosis. Median survival was no better for patients with GBMs who received chemotherapy (15 mo) than for those who did not (19 mo).

In an attempt to correct for the variability in risk factors within our relatively small population, we have classified all our newly diagnosed glioma patients by the prognostic class presented by Curran et al. (6), which takes into account age, Karnofsky score, tumor histology, neurological and mental statuses, and treatment variables (*Table 2*). This is based on a sample of 1578 patients who received radiation/chemotherapy in three recent malignant glioma study group trials. Five of our six young patients who had AAs but no neurological

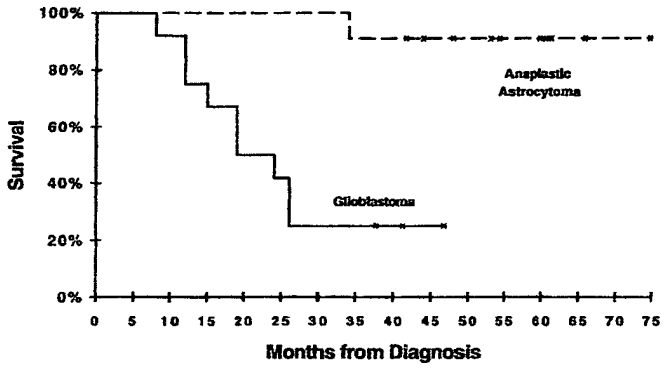


**FIGURE 2. Poly-ICLC in malignant gliomas. Kaplan-Meier survival from diagnosis (patients receiving poly-ICLC two to three times weekly). Patients with GBMs: n = 12, 3 remain alive. Patients with AAs: n = 11, 10 remain alive. *X*, cens red (live) patients.**

abnormalities (Class 1) and who received poly-ICLC are alive and well, with a median progression-free follow-up of 61 months versus an expected historical median *survival* of 59 months on standard radiation/chemotherapy (6). Most of our Class 3 patients (mostly young patients with AAs with mental status changes or young patients with GBMs) who received poly-ICLC are still alive, with a median survival or follow-up of 40 months versus an historical median survival of 18 months. Median survival for our Class 5 patients (older patients with GBMs) who received poly-ICLC two to three times weekly is 19 months versus historical survival of 9 months. However, survival of Class 5 patients who received poly-ICLC only once weekly was about the same as standard treatment. For all Class 5 patients combined, it was 13.5 months. Two-year survival for Class 3 patients receiving poly-ICLC was 80%, versus 35% on standard therapy.

Gadolinium-enhancing tumor, as revealed by MRI, has responded or remained stable for at least 6 months from study entry in 66% of the patients (20 of 30 patients) receiving poly-ICLC two to three times weekly (10 of 10 patients with AAs, 8 of 12 patients with GBMs, and 2 of 8 patients with recurrent gliomas). The best single response is given for each patient in *Table 1*. The anatomic responses for all patients who were newly diagnosed with AAs were as follows: 2 of 11, CR; 6 of 11, PR; and 3 of 11, S. The responses for all patients who were newly diagnosed with GBMs were as follows: 1 of 17, CR; 2 of 17, PR; and 6 of 17, S. Median single best tumor volume regression was 93% for patients achieving CR or PR and 65% for responding or stable patients combined. Overall responses revealed by MRI or stable rates achieved by poly-ICLC administration are 71% for a dosage of 10 mcg/kg poly-ICLC administered twice weekly (n = 7), 65% for 20 mcg/kg two or three times weekly (n = 23), and 33% for 10 or 50 mcg/kg weekly (n = 6). Of 20 responding or stable patients with MRI follow-up examinations, 12 remained stable or showed further tumor regression over ≤52 months (*Fig. 1*). Two patients, whose lesions had grown at 3 to 6 months, showed tumor regression at 6 to 12 months after receiving poly-ICLC alone (Patients 25 and 34).

Serum OAS activity increased by at least 300% in response to poly-ICLC in 24 of 29 patients tested (*Table 1*). Absolute values ranged from 0 to 135 pcgm/dl at baseline, with increases as large as 400-fold. (Separate studies have shown a clinical antiviral response to be associated with serum OAS increase of >300% after IFN treatment of hepatitis patients.) There was an association between OAS response to poly-ICLC and tumor response or stabilization, as revealed by MRI ($P$ = 0.03, Fischer's exact test). Baseline serum IFN ranged from <5 to 126 IU, with increases of 5- to 25-fold in only 4 of 15 patients. There was no relation between serum IFN and OAS activity or tumor response. Serum neopterin and IL-2 responses were highly variable and inconsistent, with median changes of only 7 and 0%, respectively. Serum TNF and IL-6 did not change at all in response to these intramuscular doses of poly-ICLC. There was no relation between serum changes in any of these cytokines and tumor response.

## DISCUSSION

The 100% sustained tumor response or stable rate and the prolonged, continuing, quality progression-free survival

**TABLE 2. Polyinosinic-Polycytidylic Acid Stabilized with Polylysine and Carboxymethylcellulose Used in the Treatment of Newly Diagnosed Malignant Gliomas: Expected and Actual Survival[a]**

| Prognostic Class[b] | No. of Patients | No. of Patients Remaining Alive | Median Age (yr) | PICLC Median Survival or Follow-up (mos) | PICLC Survival (2 yr) (%) | Historical Median Survival (mo) | Historical Survival (2 yr) (%) |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 5 | 32 | 61 | 100 | 59 | 76 |
| 2 | 1 | 1 | 54 | 46 | – | 37 | 68 |
| 3 | 10 | 6 | 34 | 40 | 80 | 18 | 35 |
| 4 | 1 | 1 | 42 | 48 | – | 11 | 15 |
| 5 | 3 | 0 | 58 | 19 | 33 | 9 | 6 |
| 5[c] | 5 | 0 | 62 | 11 | 0 | 9 | 6 |
| 6 | 3 | 0 | 70 | 12 | 0 | 5 | 4 |

[a] PICLC, polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose; –, not applicable.

[b] Prognostic class presented by Curran et al. (6). Historical survival figures are based accordingly. Poly-ICLC survival (or median follow-up for live patients) is given for each prognostic class.

[c] Class 5 patients receiving poly-ICLC only once weekly.

(present median follow-up, 54 mo) in our patients with AAs receiving poly-ICLC contrasts favorably with the expected median survival of ~26 months for patients with AAs who are undergoing standard treatment (35, 36). The median survival of 19 months for our patients with GBMs who were receiving at least twice weekly poly-ICLC is also encouraging (*Fig.* 2). However, the present pilot study is too small to provide a reliable measure of efficacy. Similarly, the low median age, relatively high Karnofsky score at entry, and relatively long interval between diagnosis and entry in some patients may have biased our sample toward natural long-term survivors, although they cannot fully explain the extent of our findings (35, 36). For example, even our subset of patients with GBMs who are older than 50 years showed a prolonged median survival to 16 months; when patients are regrouped by prognostic class considering age, clinical status, histology, and treatment, poly-ICLC survival or follow-up figures in all prognostic groups still compare favorably with those of a cohort of 1578 patients on standard radiation/chemotherapy (*Table* 2) (6).

Interpretation of anatomic response rates (as determined by MRI) in malignant glioma studies remains controversial. The initial anatomic response rates of our patients treated with poly-ICLC compare favorably with other studies, yet it is perhaps more notable that all but nine responders receiving continuous poly-ICLC have shown further tumor regression or stabilization, as revealed by MRI, at 12 to 52 months from entry, with or without concurrently administered CCNU (*Fig.* 1). We also emphasize that these patients may have unmeasurable nonenhancing tumor, and conversely, that certain areas of enhancement may represent postoperative or radiation change. The observed responses may also have been caused by radiation and/or chemotherapy. Shrinkage of enhancing lesion can be seen for months after radiation alone, but half of our responders were at least 3 months from completion of radiation therapy at study entry and were probably beyond the principal radiation effect on tumor size at the time of follow-up, 6 to 12 months later. Likewise, corticosteroid treatment cannot explain these changes, because most responders were off steroids or on a taper at the time of response. A synergistic effect between poly-ICLC and CCNU is also possible, although there was no significant difference in tumor response or survival between our patients who received CCNU and those who did not. However, most of our patients with AAs received at least one cycle of CCNU. Based on this limited sample, the optimum dose of poly-ICLC seems to be ~20 mcg/kg, administered two or three times weekly, but response that can be detected by MRI can be delayed by 6 to 12 months for some patients.

There are at least three interrelated systems stimulated by poly-ICLC, any of which, alone or in combination, might be responsible for our findings. The first two are its induction of IFN and its broad immune-enhancing effect (21). However, the levels of serum IFN induced by our doses of poly-ICLC are relatively low and have not been associated with antitumor action. Low-dose poly-ICLC also has a direct immune-enhancing action independent of IFN, including increased antibody response to antigen, and NK-cell, T-cell, macrophage, and cytokine activation. Although discussion of the complex immunostimulatory effects of poly-ICLC and the IFNs is beyond the scope of this report, their role in the potential antineoplastic effect we have seen needs further investigation. An interaction with the possible immunosuppressive role of TGF-$\beta$ in gliomas may be of particular interest (27). However, preliminary laboratory results regarding our patients show no clear relationship between tumor response and measurable serum IFN, TNF, IL-2, IL-6, or neopterin; this agrees with prior animal studies (2).

The third action of poly-ICLC is a more direct antiviral and perhaps antineoplastic effect mediated by at least two IFN-inducible nuclear enzyme systems, the 2',5'-OAS and the phosphorylated eukaryotic initiation factor 2-$\alpha$ kinase, also known as the dsRNA-dependent $P_{68}$ protein kinase ($P_{68}$ PK) (*Fig.* 3) (4, 12, 33). dsRNA induces an antiviral state in cells by functioning as an obligatory cofactor for OAS, which activates ribonuclease L, as well as for the $P_{68}$ PK, which inhibits initiation of protein synthesis. Both are very sensitive to dsRNA dose and structure (28). For example, simple, long-chain dsRNA (as in poly-ICLC) is the most potent stimulator of OAS and $P_{68}$ PK, whereas mismatched or irregular dsRNA can be inhibitory. Similarly, the $P_{68}$ PK has both high- and low-affinity binding sites and is inhibited by too high a dose of dsRNA (9). Clinically, the OAS response is also maximal at a dose of ~30 mcg/kg poly-ICLC and is much diminished above 100 mcg/kg (Kende M, Bernton N, unpublished observations). If further studies confirm our hypothesis that OAS and/or $P_{68}$ PK may mediate the possible antitumor action of poly-ICLC, this might help explain why the high doses of poly-ICLC used in early cancer trials were relatively ineffective.

The clinical half-life of the OAS response to intramuscularly administered poly-ICLC is ~2.5 days, suggesting an optimum dose schedule of two or three times per week (26) (Kende M, Bernton N, unpublished observations). Our patients showed a ≤40-fold increase in serum OAS product in response to treatment at 10–20 mcg/kg, and a significant association of serum OAS with tumor response ($P = 0.03$). However, the extent to which serum OAS reflects actual OAS (or $P_{68}$ PK) activity in the tumor or the brain is unknown, as is the extent of poly-ICLC penetration into tumor cells.

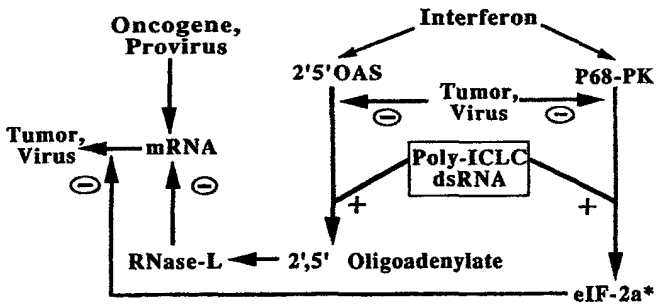


**FIGURE 3. Hypothetical antiretroviral and antitum r action of poly-ICLC. *eIF2-a**, phosphorylated eukaryotic initiati n factor 2-$\alpha$ (see text).**

The inhibition of EFC-2 glioma cells in vitro by IFN-$\beta$ is significantly associated with activation of both the OAS and $P_{68}$ PK (31). Koromilas et al. (14) demonstrated that expression of a functionally defective mutant of $P_{68}$ kinase results in malignant transformation in vitro, suggesting an important role for this enzyme in suppression of tumorigenesis. They also suggest a possible relation to the P53 tumor suppressor associated with the multiple-malignancy Li-Fraumeni syndrome, which includes astrocytomas, sarcomas, and lung and breast cancers (25).

Several viruses, including adenovirus and the human HIV, circumvent host defenses by down-regulating OAS and/or $P_{68}$ kinase, and this effect can be reversed in vitro by exogenous dsRNA (12, 34). dsRNA may be clinically useful in HIV infection (3, 32), and in vitro studies have shown an antiretroviral effect of poly-IC and poly-ICLC that is not blocked by anti-IFN antibody and is accompanied by elevation of OAS (10, 40); HIV replication is virtually halted in infected monocytes, although provirus remains present, suggesting induction of latency by poly-ICLC. Comparison with this HIV model suggests that the principal action of poly-ICLC in gliomas may be *tumoristatic* rather than *tumoricidal*; this is consistent with the delayed tumor response we see clinically. A hypothetical block of $P_{68}$ PK and/or OAS-mediated IFN action might also explain the variable response to IFNs seen in neoplastic disease, but whether malignant gliomas and certain other neoplasms use this or a similar mechanism to circumvent host defenses and whether it might also be reversible clinically by poly-ICLC remain uncertain.

Finally, recent studies have demonstrated a progression of glioma genetic abnormalities with increasing malignancy. Changes in the P53 genes on Chromosome 17 and in the 9p22 IFN complex are seen in AAs, whereas GBMs demonstrate additional changes in 9p, as well as loss of Chromosome 10 and/or amplification of the epidermal growth factor receptor gene (5, 16). The differential response we have observed between AAs and GBMs might also be explained if poly-ICLC action were to compensate clinically for the IFN gene defects on 9p but not for the changes on Chromosome 10 and elsewhere.

In conclusion, we have demonstrated the safety and tolerance of long-term, low-dose intramuscularly administered poly-ICLC in patients with malignant glioma and have tentatively identified a potentially beneficial dose range (20 mcg/kg, administered two to three times weekly). Although these findings are encouraging, we must again emphasize that any further statement regarding efficacy against malignant gliomas or other neoplasms must await controlled multicenter trials. These results also point out the need for further laboratory studies of the potential antineoplastic role of long-term, low-dose dsRNA, including the possible roles of OAS and $P_{68}$ PK in the biology of malignant gliomas and their relation to the P53 tumor suppressor.

## ACKNOWLEDGMENTS

We thank the residents and staff of the Neurology, Neurosurgery, Oncology, and Neuroradiology Services at Walter Reed Army Medical Center; Bahman Jabbari, M.D., Frazier Henderson, M.D., Joyce Olesson, R.N., Renee Smith, R.N., Donald Downey, L.P.N., and Denise Brown for help with the care of the patients; Dr. Askold Mosijczuk for critical commentary on the manuscript; and Patricia West and Ruth Ogundepo for manuscript preparation.

The views expressed herein are those of the authors and not of the Department of Defense.

**Received,** July 11, 1995.
**Accepted,** December 8, 1995.
**Reprint requests:** Andres M. Salazar, M.D., Director, Defense and Veterans Head Injury Program, Building 7, Room 224, Walter Reed Army Medical Center, Washington, DC, 20307-5000.

## REFERENCES

1. Bever C, Salazar AM, Neeley E, Ferraracio B, McFarland H, McFarlin D: Preliminary trial of poly-ICLC in chronic progressive multiple sclerosis. **Neurology** 36:489–493, 1986.
2. Black PL, Hartmann D, Pennington R, Phillips H, Schneider M, Tribble HR, Talmadge JE: Effect of tumor burden and route of administration on the immunotherapeutic properties of polyinosinic-polycytidylic acid stabilized with poly-L-lysine in carboxymethyl cellulose [poly(I,C)-LC]. **Int J Immunopharmacol** 14:1341–1353, 1992.
3. Carter WA, Strayer DR, Brodsky I, Lewin M, Pellegrino MG, Einck L, Henriques HF, Simon G, Parenti DM, Scheib RG, Schulof RS, Montefiori DD, Robinson WE, Mitchell WM, Volsky DJ, Paul D, Paxton H, Meyer WA III, Kariko K, Reichenbach N, Suhadolnik RJ, Gillespie DH: Clinical, immunological, and virological effects of ampligen, a mismatched double-stranded RNA, in patients with AIDS or AIDS-related complex. **Lancet** 1:1286–1292, 1987.
4. Chebath J, Revel M: The 2-5 A system: 2-5 A synthetases, isospecies and functions, in Baron S, Copenhaver DH, Dianzani F, Fleishman WR, Hughes TK, Klimpel GR, Niesel DW, Stanton GJ, Tyring SK (eds): *Interferons: Principles and Medical Applications.* Galveston, University of Texas Medical Branch, 1992, pp 225–236.
5. Collins VP, James CD: Gene and chromosomal alterations associated with the development of human gliomas. **FASEB J** 7:926–930, 1993.
6. Curran WJ Jr, Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, Chang CH, Rotman M, Asbell SO, Krisch RE, Nelson DF: Recursive partitioning analysis of prognostic factors in three radiation therapy oncology group malignant glioma trials. **J Natl Cancer Inst** 85:704–710, 1993.
7. Droller MJ: Immunotherapy of metastatic renal cell carcinoma with polyinosinic-polycytidylic acid. **J Urol** 137:202–206, 1987.
8. Ewel CH, Urba WJ, Kopp WC, Smith JW II, Steis RG, Rossio JL, Longo DL, Jones MJ, Alvord WG, Pinsky CM, Beveridge JM, McNitt KL, Creekmore SP: Polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose in combination with interleukin-2 in patients with cancer: Clinical and immunological effects. **Cancer Res** 52:3005–3010, 1992.
9. Galabru J, Katze MG, Robert N, Hovanessian AG: The binding of dsRNA and adenovirus VA1 RNA to the interferon-induced protein kinase. **Eur J Biochem** 178:581–589, 1989.
10. Gendelman HE, Friedman RM, Steve J, Baca LM, Turpin JA, Dveksler G, Meltzer MS, Dieffenbach C: A selective defect of interferon-alpha production in human immunodeficiency virus infected monocytes. **J Exp Med** 172:1433–1442, 1990.

11. Hawkins MJ, Levin M, Borden EC: An ECOG Phase I-II pilot study of poly-ICLC in patients with metastatic malignant melanoma. **J Biol Resp Modif** 4:664–668, 1985.
12. Katze MG: The war against the interferon-induced dsRNA-activated protein kinase: Can viruses win? **J Interferon Res** 12: 241–248, 1992.
13. Kornblith P, Walker M: Chemotherapy for malignant gliomas. **J Neurosurg** 68:1–17, 1988.
14. Koromilas AE, Roy S, Barber GN, Katze MG, Sonenberg N: Malignant transformation by a mutant of the IFN-inducible dsRNA-dependent protein kinase. **Science** 257:1685–1689, 1992.
15. Krown SE, Kerr D, Stewart WE, Field AK, Oettgen HF: Phase I trials of poly-ICLC in advanced cancer. **J Biol Resp Modif** 4:640–649, 1985.
16. Lang FF, Miller DC, Koslow M, Newcomb EW: Pathways leading to glioblastoma multiforme: A molecular analysis of genetic alterations in 65 astrocytic tumors. **J Neurosurg** 81:427–436, 1994.
17. Leibel SA, Sheline GE: Radiation therapy for neoplasms of the brain. **J Neurosurg** 66:1–22, 1987.
18. Levin VA, Prados MR, Wara WM, Davis RL, Gutin PH, Phillips TL, Lamborn KL, Wilson CB: Radiation therapy and BDU, chemotherapy followed by procarbazine, lomustine, and vincristine for the treatment of anaplastic gliomas. **Int J Radiat Oncol Biol Phys** 32:75–83, 1995.
19. Levy HB: Interferon and interferon inducers in the treatment of neoplastic diseases, in Brodsky I, Kahn SB, Moyer JH (eds): Cancer Chemotherapy II. Orlando, Grune and Stratton, 1972, pp 53–61.
20. Levy HB: Clinical studies with polynucleotides, in Baron S, Dianzani F, Stanton GJ, Fleishman WR (eds): *The Interferon System: A Current Review to 1987*. Austin, University of Texas Press, 1987, pp 469–475.
21. Levy HB: Effects of polynucleotides in monkeys and man, in Revel M (ed): *Clinical Aspects of Interferon*. Boston, Kluwer, 1988.
22. Levy H, Salazar AM: Interferon inducers, in Baron S, Copenhaver DH, Dianzani F, Fleishman WR, Hughes TK, Klimpel GR, Niesel DW, Stanton GJ, Tyring SK (eds): *Interferon: Principles and Medical Applications*. Galveston, University of Texas Medical Branch, 1992, pp 65–76.
23. Levy HB, Baer C, Baron S, Buckler CE, Gibbs CJ, Iadarola MJ, London WT, Rice J: A modified polyribo-inosinic-polyribocytidylic acid complex that induces interferon in primates. **J Infect Dis** 132:434, 1975 (abstr).
24. Mahaley MS, Bertsch L, Cush S, Gillespie GY: Systemic gamma interferon therapy for recurrent gliomas. **J Neurosurg** 69:826–829, 1988.
25. Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH: Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. **Science** 250:1233–1238, 1990.
26. Maluish AE, Reid JW, Crisp EA, Overton WR, Levy H, Foon KA, Herberman RB: Immunomodulatory effect of poly-ICLC in cancer patients. **J Biol Resp Modif** 4:656–663, 1985.
27. Maxwell M, Galanopoulos T, Neville-Golden J, Antoniades HN: Effect of the expression of transforming growth factor-B2 in primary human glioblastomas on immunosuppression and loss of immune surveillance. **J Neurosurg** 76:799–804, 1992.
28. Minks M, West DK, Benvin S, Baglioni C: Structural requirements of dsRNA for the activation of 2',5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. **J Biol Chem** 254: 10180–10183, 1979.
29. Nakamura O, Shitara N, Matsutani M, Takakura K, Machida H: Phase I-II trials of poly-ICLC in malignant brain tumor patients. **J Interferon Res** 2:1–4, 1982.
30. Rettenmaier MA, Berman ML, DiSaia PJ: Treatment of advanced ovarian cancer with poly-ICLC. **Gynecol Oncol** 24:359–361, 1986.
31. Rosenblum MG, Yung WKA, Kelleher PJ, Ruzicka F, Steck PA, Borden EC: Growth inhibitory effects of interferon-$\beta$ but not interferon-$\alpha$ on human glioma cells: Correlation of receptor binding, 2',5'-oligoadenylate synthetase and protein kinase activity. **J Interferon Res** 10:141–151, 1990.
32. Salazar AM, Morales J, Levy HB, de Jesus C, Sarin P, Hawkes C, Martin A: Intramuscular poly-ICLC in HIV infection: Preliminary findings. **Neurology** 40[Suppl 1]:238, 1990 (abstr).
33. Samuel CE: The RNA-dependent P1/eIF-2a protein kinase, in Baron S, Copenhaver DH, Dianzani F, Fleishman WR, Hughes TK, Klimpel GR, Niesel DW, Stanton GJ, Tyring SK (eds): *Interferon: Principles and Medical Applications*. Galveston, University of Texas Medical Branch, 1992, pp 237–249.
34. Schröder HC, Wenger R, Kuchino Y, Müller WEG: Modulation of nuclear matrix-associated 2',5'-oligoadenylate metabolism and ribonuclease L activity in H9 cells by human immunodeficiency virus. **J Biol Chem** 264:5669–5673, 1989.
35. Shapiro WR, Green SB, Burger PC, Mahaley MS, Selker RG, Van Gilder JC, Robertson JT, Ransohoff J, Mealey J, Strike TA, Pistenmaa DA: Randomized trial of three chemotherapy regimens and two radiotherapy regimens in postoperative treatment of malignant glioma. **J Neurosurg** 71:1–9, 1989.
36. Shapiro WR, Green SB, Burger PC, Selker RG, VanGilder JC, Robertson JT, Mealey J Jr, Ransohoff J, Mahaley MS Jr: A randomized comparison of intra-arterial versus intravenous BCNU, with or without intravenous 5-fluorouracil, for newly diagnosed patients with malignant glioma. **J Neurosurg** 76:772–781, 1992.
37. Talmadge JE, Hartmann D: Optimization of an immunotherapeutic protocol with poly-ICLC. **J Biol Resp Modif** 4:484–489, 1985.
38. Talmadge JE, Herberman RB, Chirigos MA, Maluish AE, Schneider MA, Adams JS, Philips H, Varesio L, Long C, Oldham RK, Wiltrout RH: Hyporesponsiveness to augmentation of murine natural killer cell activity in different anatomical compartments by multiple injections of various immunomodulators, including recombinant interferons and interleukin-2. **J Immunol** 135:2483–2491, 1985.
39. Theriault RL, Hortobagye GN, Budzar AU, Levy HB, Hersh EM: Evaluation of poly-ICLC in metastatic breast cancer. **Cancer Treat Rep** 70:1341–1342, 1986.
40. Ushijima H, Rytik PG, Schäcke H, Scheffer U, Müller WEG, Schröder HC: Mode of action of the anti-AIDS compound poly(I) · poly($C_{12}$U) (ampligen): Activator of 2',5'-oligoadenylate synthetase and double-stranded RNA-dependent kinase. **J Interferon Res** 13:161–171, 1993.
41. Walker MD, Alexander E, Hunt WE, MacCarty CS, Hamaley MS, Mealey J, Norrell HA, Owens G, Ransohoff J, Wilson CB, Gehan EA, Strike TA: Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. **J Neurosurg** 49:333–343, 1978.


## COMMENTS

This article considers the pilot use of polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) in the treatment of newly diagnosed and recurrent malignant gliomas. The inherent problems of small numbers and sequential accrual have been addressed, and the results seem compelling, encouraging the institution of Phase II/III clinical trials. Although some issues can be made with

using heterogenous historical controls, the description of median survival in this population of 18 months in newly diagnosed glioblastomas (GBMs) multiforme and 12 months from recurrence in recurrent malignant gliomas is compelling. The question of age bias, in that the best responses were seen in the youngest patients, cannot be adequately ruled out in the study of this design, but it seems that further clinical trials are well warranted with this agent.

**Tom Mikkelsen**
*Detroit, Michigan*

This is an interesting report of a Phase I-II dose escalation trial of the immunomodulator and antiviral agent, poly-ICLC (20 mg/kg, administered two to three times weekly) in the treatment of 38 patients with malignant gliomas. Twenty-nine patients (11 with anaplastic astrocytomas [AAs] and 18 with GBMs) were treated adjuvantly after external beam radiation therapy; 9 patients (with GBMs and AAs) were treated at the time of recurrence. Twenty-three of 38 patients received nitrosourea concurrently, including 10 of 11 patients in the AA group. The authors report a 52-month median survival in the AA group, compared to an expected median survival of 26 months. The authors also analyze their data according to a prognostic model presented by Curran et al. (1), which is an important new approach. The authors have eliminated two patients from analysis in the GBM group because those two patients dropped out of the study (one because of side effects and the other for personal reasons). Those patients probably had a decreased median survival (≤12 mo), and their inclusion in this trial, in an "intent to treat" analysis, would probably change median survival to ~15 months. In reviewing the response of malignant gliomas to poly-ICLC, we must reexamine the small number of patients, the concurrent administration of lomustine, and the great variability of individual response in young patients before drawing major conclusions about the data presented. The authors acknowledge this and discuss the possibility of a future randomized trial to assess the therapeutic effect of poly-ICLC. They have established the safety and tolerance of poly-ICLC.

**Harry S. Greenberg**
*Ann Arbor, Michigan*

This article is about the effects of an old compound, poly-ICLC, administered intramuscularly at a new dosage schedule. The authors demonstrate the activity of poly-ICLC on AAs and GBMs. At doses lower than those used a decade ago, response rates for both diseases, including stable disease, were impressive. Although dosage several times per week seems to be superior to a single weekly dose, the design of the study does not permit a firm conclusion. The authors adopted a cautious approach to their data in a relatively small number of patients, presenting historical data but recognizing the limitations of comparisons with such data. This pilot study should provide an impetus that will lead to a controlled, multicenter trial.

**Malcolm S. Mitchell**
*San Diego, California*